Cell Metabolism
Review

Metformin: From Mechanisms of Action to Therapies

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Metformin is currently the first-line drug treatment for type 2 diabetes. Besides its glucose-lowering effect, there is interest in actions of the drug of potential relevance to cardiovascular diseases and cancer. However, the underlying mechanisms of action remain elusive. Convincing data place energy metabolism at the center of metformin's mechanism of action in diabetes and may also be of importance in cardiovascular diseases and cancer. Metformin-induced activation of the energy-sensor AMPK is well documented, but may not account for all actions of the drug. Here, we summarize current knowledge about the different AMPK-dependent and AMPK-independent mechanisms underlying metformin action.

Introduction

Metformin has been an important drug for treatment of type 2 diabetes (T2D) for decades. It is the most widely used oral antihyperglycemic agent and is currently recommended as first line therapy for all newly diagnosed T2D patients (American Diabetes Association, 2014). Metformin (N, N-dimethylbiguanide) belongs to the biguanide class of antidiabetic drugs (containing two linked guanidine rings) originally derived from galegine (isoamylene guanidine), a guanidine derivative found in the French lilac *Galega officinalis*. Among the three biguanides developed for diabetes therapy, metformin has a superior safety profile, and it is well tolerated. Two other biguanides, phenformin and buformin, were withdrawn in the early 1970's due to risk of lactic acidosis and increased cardiac mortality. The incidence of lactic acidosis with metformin at therapeutic doses is rare (less than three cases per 100.000 patient-years) and not greater than with non-metformin therapies. Metformin has been used widely in the treatment of T2D for over 50 years and has been found to be safe and efficacious both as monotherapy and in combination with other oral antidiabetic agents and insulin. It offers the major clinical advantage of not inducing hypoglycemia or weight gain and ameliorates hyperglycemia with remarkable cardiovascular safety. Besides its use in T2D, there is interest in the use of metformin for the treatment of polycystic ovary disease, diabetic nephropathy, and gestational diabetes (Viollet et al., 2012). The drug also has the advantage of counteracting the cardiovascular complications associated with diabetes as reported in a large cohort of individuals from the United Kingdom Prospective Diabetic Study (UKPDS) (1998). Another possible benefit for metformin use is the association with decreased cancer risk and improved cancer prognosis (Pollak, 2012b; Viollet et al., 2012). Although metformin has been used in Europe for treatment of hyperglycemia since 1957 (and in the USA since FDA approval in 1994), the exact molecular mechanisms of its therapeutic action remain obscure. In this review, we summarize what is currently known about these molecular mechanisms in the context of classic use for T2D and also for possible novel areas of therapeutic application.

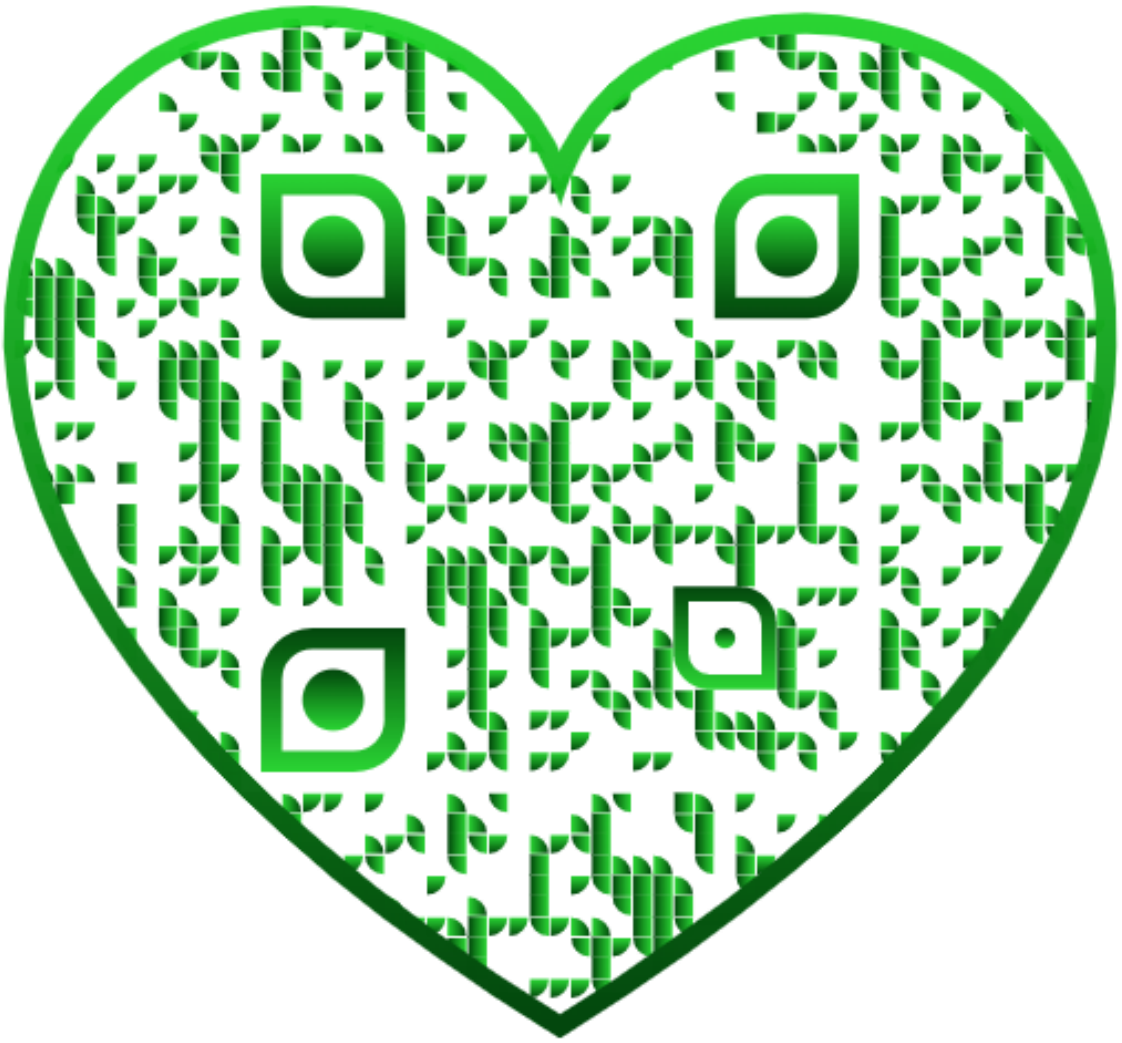
Pharmacokinetics and Pharmacogenomics

Pharmacokinetics of Metformin

The optimal oral metformin dose for many diabetic patients is ~2 g/day. After a single oral dose, metformin is rapidly distributed to many tissues following partial absorption by the small intestine, but the luminal concentration in the gastrointestinal tract remains high. The peak plasma concentration occurs in 3 hr (increasing from 1.0 to 1.6 μg/ml [about 6 to 10 μM] after a 0.5 g dose and to ~3 μg/ml [about 18 μM] after a 1.5 g dose) with a mean plasma half-life of about 20 hr (Tucker et al., 1981). When the human metformin dose of 20 mg/kg/day orally is translated to the mouse equivalent dose of 250 mg/kg/day, according to the normalization to body surface area, murine plasma levels of metformin of up to 1.7 μg/ml (about 10 μM) are achieved (Memmott et al., 2010). This is in the range achieved when conventional antidiabetic doses are used in humans (Tucker et al., 1981). Biodistribution studies in mice using ^{14}C-labeled metformin showed accumulation mainly in the gastrointestinal tract, kidney, and liver (Wilcock and Bailey, 1994). It is important to note that being supplied directly by blood coming from the portal vein, the liver may contain a concentration of orally administered metformin substantially higher than in the general circulation and other organs (Wilcock and Bailey, 1994). Metformin liver concentrations of greater than 180 μmol/kg wet weight and 250 μmol/kg wet weight in normal and diabetic rodents, respectively, can be achieved after a single dose of 50 mg/kg (Wilcock and Bailey, 1994).

Cellular Uptake of Metformin

Metformin is an unusually hydrophilic drug that mostly exists in a positively charged protonated form under physiological conditions. These physicochemical properties make rapid and passive diffusion through cell membranes unlikely. Indeed, transport of metformin involves an active uptake process via solute carrier organic transporters. The intestinal absorption of metformin is primarily mediated by the plasma membrane monoamine transporter (PMAT, *SLC29A4* gene), which is localized on the luminal side of enterocytes. Organic cation transporter 1 (Oct1, *SLC22A1* gene) is expressed on the basolateral membrane of



enterocytes and may be responsible for the transport of metformin into the interstitial fluid (Gong et al., 2012). The primary mediator of hepatic metformin uptake is OCT1 and possibly OCT3 (*SLC22A3* gene), expressed at the basolateral membrane of hepatocytes (Gong et al., 2012). The clearance of metformin is dependent on renal elimination, as metformin does not undergo relevant biotransformation in the liver or biliary excretion. In the kidney, metformin is taken up into renal epithelial cells by OCT2 (*SLC22A2* gene), expressed on the basolateral membrane, and excreted into the urine via multidrug and toxin extrusion 1 and 2 (MATE1 gene SLC47A1 and MATE2 gene SLC47A2).

Pharmacogenomics of Metformin

Considerable interindividual heterogeneity in clinical efficacy and the pharmacokinetic disposition of metformin has been reported in the treatment of diabetic patients. This may be explained by variability in genetic polymorphisms of cation transporters. It was first reported that individuals carrying polymorphisms of the OCT1 gene *SLC22A1* display an impaired effect of metformin in lowering blood glucose levels, consistent with the great reduction of hepatic metformin uptake observed in *OCT1*$^{-/-}$ mice (Shu et al., 2007). However, these results have not been confirmed in the long-term follow-up of a large observational cohort of patients treated with metformin (Zhou et al., 2009). Conversely, variants in the MATE1 gene *SLC47A1* enhance the effect of metformin on glycated hemoglobin (HbA1c) and glucose tolerance in T2D patients (Becker et al., 2009). In *MATE1*$^{-/-}$ mice, urinary excretion of metformin is significantly decreased, suggesting that MATE1 is essential for renal clearance of the drug (Tsuda et al., 2009). Among new candidate genetic determinants of metformin response, single nucleotide polymorphisms have been identified in the AMPK subunit genes, *PRKAA1*, *PRKAA2* and *PRKAB2*, and the LKB1 gene *STK11* (Jablonski et al., 2010). In addition, a recent genome-wide association study showed association between a large locus on chromosome 11, encompassing several genes, and glycemic variability in response to metformin therapy (Zhou et al., 2011). This locus includes the ataxia telangiectasia mutated (ATM) gene, and it was suggested as the most likely candidate given its association with insulin resistance and T2D. However, additional studies are needed to clearly delineate genetic influences on the clinical response to metformin.

Metformin and Mitochondrial Oxidative Phosphorylation

It is generally accepted that actions of metformin (and other biguanides) on mitochondria underlie most of the pleiotropic effects of the drug. This emerging consensus originates from two seminal papers published in 2000 reporting that metformin decreases cellular respiration by a mild and specific inhibition of the respiratory-chain complex 1 (NADH:ubiquinone oxidoreductase) without affecting any other steps of the mitochondrial machinery (El-Mir et al., 2000; Owen et al., 2000). However, reviewing early mechanistic studies, the first observation reporting effects of biguanide derivatives on mitochondrial oxidative phosphorylation (OXPHOS) comes from the pioneering work of Hollunger in the 1950s that linked the increase in glycolysis induced by this class of molecules to the inhibition of cellular respiration (Hollunger, 1955). Later, high concentrations of various biguanide derivatives were found to specifically reduce NADH but not succinate oxidation in submitochondrial particles, assigning the inhibitory effect of these compounds on OXPHOS to the complex 1 of the mitochondrial electron transport chain (ETC). Thus, the concept that members of the biguanide family, including phenformin and metformin, exert many of their actions though modulation of mitochondrial energetics is not a recent proposal. During the last decade, the specific inhibition of the mitochondrial respiratory-chain complex 1 by metformin was confirmed in many cellular models, including rat, mouse and human primary hepatocytes (El-Mir et al., 2000; Owen et al., 2000; Stephenne et al., 2011), hepatoma, and adrenocortical carcinoma immortalized cell lines (Guigas et al., 2004; Hirsch et al., 2012; Kim et al., 2013), skeletal muscle homogenates (Brunmair et al., 2004), endothelial cells (Detaille et al., 2005), pancreatic beta cells (Hinke et al., 2007), neurons (El-Mir et al., 2008), peripheral blood mononuclear cells and platelets (Piel et al., 2014), and more recently in cancer cells (Bridges et al., 2014; Janzer et al., 2014; Scotland et al., 2013; Wheaton et al., 2014). It has been reported that this transient inhibition of complex 1 induces a drop in cellular energy charge, a measure of the energetic state of the cell defined as ([ATP] + 0.5[ADP])/([ATP] + [ADP] + [AMP]) (Foretz et al., 2010; Stephenne et al., 2011). The resulting fall in cellular ATP concentration and an increase in both ADP/ATP and AMP/ATP ratios activates the AMP-activated protein kinase (AMPK), a critical energy sensor of cellular energy homeostasis that integrates multiple signaling networks to coordinate a wide array of compensatory, protective, and energy-sparing responses (Viollet et al., 2012).

Although the exact mechanism(s) by which metformin inhibits complex 1 remains unknown, some studies were unable to detect a direct effect on isolated mitochondria except at very high concentrations, suggesting that the mitochondrial action of the drug requires intact cells (El-Mir et al., 2000; Guigas et al., 2004). On the other hand, it was recently reported that metformin (and other biguanides) can directly inhibit function of purified respiratory-chain complex 1, as well as in isolated mitochondria and submitochondrial particles from bovine heart (Andrzejewski et al., 2014; Bridges et al., 2014), in line with previous data reporting similar properties using very high concentrations (20–100 mM) of the drug (Dykens et al., 2008; Owen et al., 2000). However, it remains to be demonstrated if these mechanisms operate in vivo.

It was also shown that the inhibitory effect of metformin on complex 1 was not prevented by nitric oxide (NO) synthase inhibitors or reactive oxygen species (ROS) scavengers (El-Mir et al., 2000) and was independent of AMPK, at least in primary mouse hepatocytes (Stephenne et al., 2011). Among the possible underlying mechanisms, it was proposed that the positive charge of metformin might account for its accumulation within the matrix of energized mitochondria, driven by the membrane potential (Owen et al., 2000). In addition, its apolar hydrocarbon side-chain would also promote its binding to hydrophobic structures, such as the constitutive phospholipids of mitochondrial membranes. Importantly, the lipophilicity of metformin, which is mostly due to its dimethyl-substituted terminal amino group, is much less than those of phenformin, which is more evenly distributed along its biguanide backbone. These peculiar physicochemical characteristics explain why two structurally related biguanides affect differently the mitochondrial machinery, metformin being a weak but specific inhibitor of complex 1 whereas phenformin exerting a more potent but less specific action on the

mitochondrial ETC (Drahota et al., 2014). It is also worth mentioning that the inhibition of complex 1 activity by metformin is rather mild when compared to the reference inhibitor rotenone (El-Mir et al., 2000). Furthermore, metformin was shown to significantly reduce mitochondrial ROS production by selective inhibition of the reverse electron flow through the respiratory-chain complex 1, whereas rotenone triggers ROS production by increasing forward electron flow (Batandier et al., 2006). Taken together, this suggests that their respective site of action on one or several of the subunits constituting the respiratory-chain complex 1 differ. Although recent studies have led to significant improvements in the understanding of its structure-function relationships (Bridges et al., 2014), many aspects of the regulation of mitochondrial complex 1, such as the so-called active/deactive transition, remain incompletely understood. Further investigations are therefore still required to clarify the mechanism(s) by which metformin modulates the respiratory-chain complex 1 in such a unique way. Interestingly, it has been reported that direct binding of metformin to mitochondrial copper ions might be crucial for the metabolic effects of the drug (Logie et al., 2012). These findings point out again the crucial involvement of mitochondria in the molecular mechanism of action of metformin. Of note, not all the effects of biguanides are mediated by mitochondria since the glucose metabolism of erythrocytes, which are devoid of this organelle, was shown to be significantly affected secondary to metformin-induced change in cell membrane fluidity induced by the drug (Muller et al., 1997).

Metformin and Treatment of Type 2 Diabetes

Metformin exerts its glucose-lowering effect primarily by decreasing hepatic glucose production through suppression of gluconeogenesis and enhancing insulin suppression of endogenous glucose production and, to a lesser extent, by reducing intestinal glucose absorption and possibly improving glucose uptake and utilization by peripheral tissues, such as skeletal muscle and adipose tissue (Natali and Ferrannini, 2006). Of note, it has been reported that metformin does not improve peripheral insulin sensitivity (Natali and Ferrannini, 2006), and improvements in insulin sensitivity in muscle may be related to the use of higher doses of metformin than clinically relevant. Additionally, metformin may also improve glucose homeostasis by interacting with the incretin axis through the action of glucagon-like peptide 1 (GLP-1) (Maida et al., 2011; Mulherin et al., 2011). A recent study has found evidence that metformin and phenformin antagonize the action of the counter-regulatory hormone glucagon to suppress hepatic glucose production (Miller et al., 2013). Furthermore, Fullerton and colleagues recently showed that metformin-induced improvements in insulin action operate through alterations in hepatic lipid homeostasis via the inhibitory phosphorylation of acetyl CoA carboxylase (ACC) by AMPK (Fullerton et al., 2013).

Inhibition of Hepatic Gluconeogenesis

An important breakthrough in the understanding of the molecular mechanism underlying metformin action was the demonstration that metformin-induced AMPK activation is associated with the inhibition of glucose production in primary hepatocytes (Zhou et al., 2001). The role for AMPK in mediating the action of metformin was initially supported by the reduction in metformin's effect on glucose production in primary hepatocytes treated with compound C (Zhou et al., 2001), an AMPK inhibitor that is now recognized to be nonselective. Thereafter, it was reported that, ablation of liver kinase B1 (LKB1, the upstream kinase that phosphorylates and activates AMPK) in the liver prevented the antihyperglycemic effects of metformin in high-fat-fed mice (Shaw et al., 2005), also supporting the involvement of the kinase in the inhibition of glucose production by the drug. In this study, it was shown that LKB1/AMPK signaling controls the phosphorylation and nuclear exclusion of the transcriptional coactivator cAMP-response element-binding protein (CREB)-regulated transcription coactivator 2 (CRTC2, also known as TORC2) (Shaw et al., 2005), a pivotal regulator of gluconeogenic gene transcription in response to fasting. In addition, AMPK activation by metformin has also been reported to be involved in the transcriptional regulation of hepatic gluconeogenic enzyme genes by different mechanisms: (i) dissociation of the CREB-CBP (CREB-binding protein)-TORC2 transcription complex, through the phosphorylation of the transcriptional coactivator CBP via atypical protein kinase C ι/λ (He et al., 2009), (ii) increased expression of the orphan nuclear receptor small heterodimer partner (Lee et al., 2010), and (iii) induction of SIRT1-mediated CRTC2 deacetylation (Caton et al., 2010). However, the impact of reduction in gluconeogenic gene expression in metformin action has been recently disputed. Forced increase in gluconeogenic enzymes expression did not counteract the metformin-induced reduction in glucose output (Foretz et al., 2010), this being in line with the emerging concept that transcriptional expression of PEPCK and G6Pase only weakly influences hepatic glucose output in patients with T2D (Samuel et al., 2009).

Over the last years, controversy has arisen concerning the involvement of AMPK in the therapeutic effects of metformin on hepatic glucose production. Indeed, although metformin activates AMPK, this may not explain all of the therapeutic effects of the drug. Recent work in liver and primary hepatocytes from knockout models for both AMPKα1/α2 catalytic subunits and the upstream activating kinase LKB1 reveal that neither AMPK nor LKB1 are essential for metformin inhibition of hepatic glucose production (Foretz et al., 2010). However, a new report challenges these results and now shows that low concentrations of metformin suppress glucose production via AMPK activation independently of an increase in the AMP/ATP ratio (Cao et al., 2014). A central question raised by this work is how metformin activates AMPK without affecting energy charge. As described above, there is a consensus that metformin activates AMPK indirectly, secondary to the inhibition of the mitochondrial respiratory-chain complex 1, leading to ATP depletion and an increase in AMP levels (Hawley et al., 2010). In addition, it has been suggested that perturbation of intracellular ATP levels, but not AMPK activation per se or inhibition of gluconeogenic gene expression, constitutes the critical factor underlying the effects of metformin on hepatic glucose output (Foretz et al., 2010). Gluconeogenesis is an energetically costly anabolic process, requiring six ATP equivalents per molecule of glucose synthesized, and it seems likely that the metformin-induced increase in AMP exerts a major role in the flux control of hepatic gluconeogenesis by the drug. Indeed, AMP is a potent allosteric inhibitor of fructose 1,6-bisphosphatase, a key enzyme in gluconeogenesis. Additionally, high AMP levels inhibit adenylate cyclase, thereby reducing cyclic AMP (cAMP) formation in response to glucagon and thus, fasting glucose levels (Miller et al., 2013).

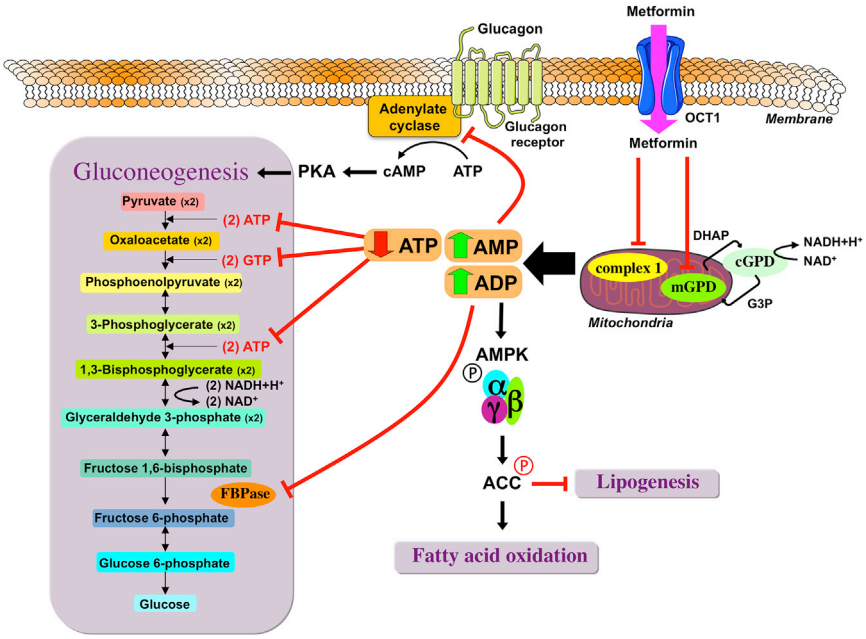


Figure 1. Molecular Mechanisms of Metformin-Induced Inhibition of Hepatic Glucose Output

Metformin is transported into hepatocytes mainly through OCT1 and partially inhibits mitochondrial respiratory-chain complex 1, resulting in reduced ATP levels and accumulation of AMP. Gluconeogenesis is reduced as a result of ATP deficit limiting glucose synthesis, increased AMP levels leading to reduced activity of the key gluconeogenic enzyme FBPase, inhibition of adenylate cyclase and cAMP-PKA signaling, and inhibition of mGPD contributing to altered redox state and reduced conversion of glycerol to glucose. Metformin-induced change in AMP/ATP ratio also activates AMPK, which suppresses lipid synthesis and exerts insulin sensitizing effects. Abbreviations: ACC, acetyl CoA carboxylase; AMPK, AMP-activated protein kinase; cAMP, cyclic AMP; complex 1, respiratory-chain complex 1; DHAP, dihydroxyacetone phosphate; FBPase, fructose-1,6-bisphosphatase; G3P, glycerol-3-phosphate; cGPD, cytosolic glycerophosphate dehydrogenase; mGPD, mitochondrial glycerophosphate dehydrogenase; OCT1, organic transporter 1; PKA, protein kinase A.

Taken together, a growing body of data indicates that multiple AMPK-independent regulatory points exist for direct AMP- and ATP-mediated effects of metformin on gluconeogenesis (Figure 1). In addition, it was very recently reported that the reduction in hepatic gluconeogenesis by metformin might result from a direct inhibition of the mitochondrial glycerophosphate dehydrogenase (mGPD), identifying another putative mitochondrial target of the drug (Madiraju et al., 2014). Inhibition of mGPD halts the glycerophosphate shuttle, blocking gluconeogenesis from glycerol and raising cytosolic NADH that feeds back on lactate dehydrogenase and also impairs incorporation of lactate into glucose.

Regulation of Lipid Metabolism

Another effect of metformin is to improve lipid metabolism by reducing hepatic steatosis as demonstrated in rodent liver (Lin et al., 2000; Woo et al., 2014) and also reported in a clinical study (Marchesini et al., 2001). It was also recently reported that metformin exerts a beneficial effect on circulating lipids by lowering plasma triglycerides, through a selective increase in VLDL-triglyceride uptake and fatty acid oxidation in brown adipose tissue (Geerling et al., 2014). The metformin-induced reduction in tissue lipid storage is consistent with an increase in both fatty acid oxidation and inhibition of lipogenesis, presumably mediated by AMPK activation (Geerling et al., 2014; Zang et al., 2004; Zhou et al., 2001). Further support for a role of AMPK in the mechanisms of metformin action on lipid metabolism was recently provided in knockin mouse models in which ACC1 and ACC2 were rendered insensitive to AMPK phosphorylation (Fullerton et al., 2013). These mice are refractory to the lipid-lowering and insulin-sensitizing effects of metformin, showing that metformin-induced reduction in blood glucose levels depends on its ability to lower cellular fatty acid levels through the AMPK-dependent phosphorylation of ACC. Thus, the inhibition of hepatic glucose production by metformin may be, at least in certain conditions, secondary to the effects of the drug on ACC. These observations offer a potential explanation for the lack of metformin action on blood glucose levels in liver-specific LKB1-knockout mice fed on a high-fat diet (Shaw et al., 2005). Indeed, impaired metformin-induced AMPK phosphorylation in the absence of LKB1 would prevent ACC phosphorylation and the ability of metformin to improve insulin sensitivity and lower blood glucose. Therefore, metformin can acutely suppress hepatic glucose output by acting on distinct metabolic pathways via AMPK-independent and AMPK-dependent mechanisms in the context of insulin resistance (Figure 1).

Metformin Action in Cardiovascular System

Metformin and Cardioprotection

Cardiovascular diseases are undoubtedly associated with T2D, and ischemic heart disease is the main cause of death in the T2D population (Grundy et al., 1999). The cardiovascular dysfunctions associated with T2D are macrovascular and microvascular abnormalities including atherosclerosis of large arteries and coronary atherosclerosis, which contributes to not only diabetes-related mortality and morbidity but also includes diabetic cardiomyopathy, a specific heart muscle dysfunction that occurs independently of coronary artery disease (Bugger and Abel, 2014). Although metformin is a first-line glucose-lowering pharmacological agent, its use was historically contraindicated in patients with heart failure due to concerns regarding increased risk of lactic acidosis. However, numerous studies revealed that metformin-associated lactic acidosis is minimal and that metformin treatment clearly reduces mortality and morbidity of type 2 diabetic patients with cardiovascular diseases such as stable coronary artery disease, acute coronary syndrome, and myocardial infarction (Eurich et al., 2013; Masoudi et al., 2005). Currently, clinical practice guidelines recommend using metformin as first-line therapy in diabetic patients with heart failure (American Diabetes Association, 2014). The UKPDS trial nicely demonstrated that metformin was more effective than sulphonylurea or insulin in patients allocated to intensive blood-glucose control (1998). In this study, metformin significantly reduced all-cause

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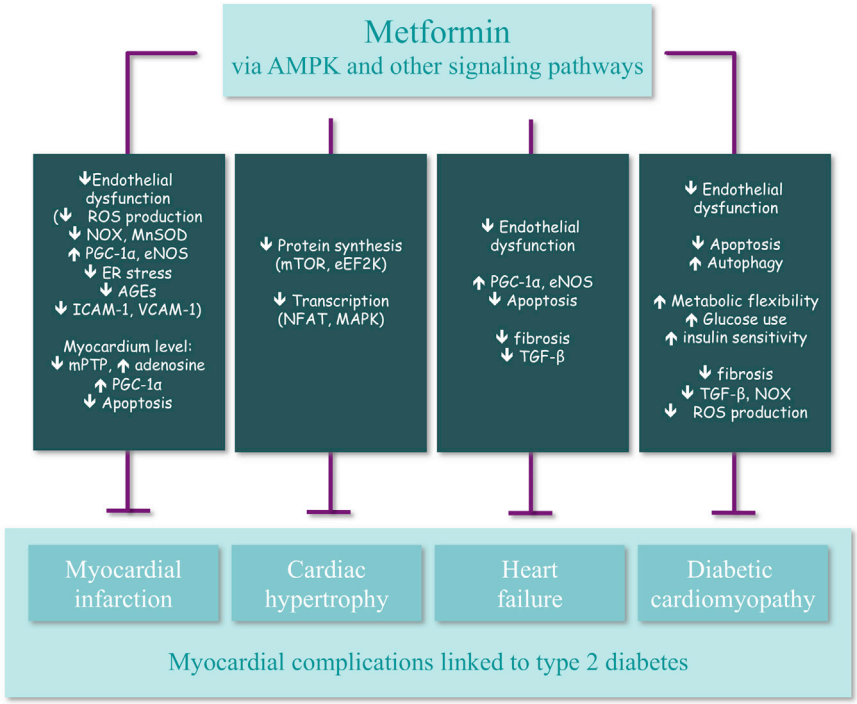

Figure 2. Main Putative Molecular Mechanisms Involved in the Cardioprotective Effects of Metformin

Animal and in vitro studies proposed a protective action of metformin against several cardiovascular diseases linked to T2D, including myocardial infarction, hypertrophy, and diabetic cardiomyopathy, which lead to cardiac dysfunction that could evolve to heart failure. The molecular mechanisms involved in this protection are multifaceted, targeting endothelial, cardiomyocyte, and fibroblast (dys)functions. More details are given in the text. Abbreviations: AGEs, advanced glycation end-products; AMPK, AMP-activated protein kinase; eEF2K, eukaryotic elongation factor 2 kinase; eNOS, endothelial nitric oxide synthase; ER, endoplasmic reticulum; ICAM-1, intercellular adhesion molecule-1; MAPK, mitogen-activated protein kinase; MnSOD, Manganese superoxide dismutase; mPTP, mitochondrial permeability transition pore; mTOR, mammalian target of rapamycin; NFAT, calcineurin-nuclear factor of activated T cells; NOX, NADPH oxidase; PGC-1α, peroxisome proliferator-activated receptor-gamma coactivator-1alpha; ROS, reactive oxygen species; TGF-β, transforming growth factor-β; VCAM-1, vascular cell adhesion molecule-1.

mortality and diabetes-related death by 36% and 42%, respectively with a significant reduction in myocardial infarction events, which persisted after a 10-year follow-up (Holman et al., 2008). Numerous recent studies have confirmed the UKPDS conclusions. In these studies, metformin use was associated with better short- and/or long-term prognosis than other antidiabetic treatments in diabetic patients with acute coronary syndrome (Hong et al., 2013) or chronic heart failure (Eurich et al., 2013). However, it has to be noted that the benefit obtained with metformin in comparison to the other glucose-lowering agents is generally quite modest. Importantly, the use of metformin in nondiabetic patients suffering from coronary heart disease was not associated with any beneficial effects (Lexis et al., 2014; Preiss et al., 2014). Several meta-analyses have been recently performed (Boussageon et al., 2012; Eurich et al., 2013; Lamanna et al., 2011). Their inclusion criteria (only population at low risk of mortality; including or not patients with heart failure; including or not nondiabetic patients) and their conclusions slightly differ. The first concludes that metformin monotherapy improves survival whereas concomitant utilization with sulphonylurea was associated with reduced survival (Lamanna et al., 2011). In the second, the effectiveness of metformin to avoid death or cardiovascular events is not established by existing studies for the second meta-analysis (Boussageon et al., 2012). Finally, the more recent meta-analysis indicates that metformin treatment is associated with reduced mortality compared with controls and is at least as safe as other glucose-lowering agents in diabetic patients with heart failure (Eurich et al., 2013). Future clinical trials are clearly necessary to definitively conclude about the safety and cardioprotective effects of metformin treatment.

Metformin in Hypertension and Atherogenesis

The mechanisms involved in the beneficial cardiovascular effects of metformin in diabetic patients are not fully understood. A plausible explanation is the systemic antihyperglycemic effect of the drug, whereby lowered gluconeogenesis decreases glucose levels and secondarily reduces insulin levels. However, several other molecular mechanisms directly targeting the cardiovascular system have been suggested by animal studies to participate in the benefits of metformin (Figure 2). A large part of these effects appears to be mediated by AMPK. The activation of AMPK by metformin-induced energy stress is relevant to the vascular system, which is known to be altered in diabetes by endothelial dysfunction and atherogenesis. Atherogenesis is accompanied by an impairment of endothelium-dependent relaxation, increased ROS production, and reduced nitric oxide (NO) bioavailability, mediating proinflammatory and prothrombotic mechanisms including platelet aggregation and leukocyte adhesion to the wall. Several cellular and animal studies have evaluated the potential antiatherogenic action of metformin and AMPK activation. Metformin has been shown to inhibit high glucose-dependent ROS overproduction in aortic endothelial cells and its consequent endothelial dysfunction (Detaille et al., 2005; Ouslimani et al., 2005). The decrease in ROS production is mediated by a double mechanism involving a reduction in NADPH oxidase activity and an inhibition of the respiratory-chain complex 1. Two more recent studies proposed that AMPK mediates this metformin-induced NADPH oxidase inactivation reducing cytoplasmic ROS production (Batchuluun et al., 2014; Bhatt et al., 2013). In parallel, Kukidome and colleagues showed that AMPK activation reduces the hyperglycemia-mediated mitochondrial ROS overproduction via the induction of manganese superoxide dismutase and the promotion of peroxisome proliferator-activated receptor-gamma coactivator-1alpha (PGC-1α)-dependent mitochondrial biogenesis in human umbilical vein endothelial cells (Kukidome et al., 2006). AMPK activation is also known to induce endothelial NO

synthase (eNOS) activation and NO-dependent vasodilation. In line with this action, it has been recently shown that metformin restores endothelial function through the inhibition of endoplasmic reticulum (ER) stress and oxidative stress and via the increase in NO bioavailability in obese diabetic mice, these effects being mediated by the AMPK/peroxisome proliferator-activated receptor δ pathway (Cheang et al., 2014). In addition, metformin targets advanced glycation end-products (AGEs), which are significant contributors of complications linked to diabetes. Indeed, metformin, independently of its antihyperglycemic property, is able to reduce AGEs synthesis and the expression of their specific cell receptor called RAGE in endothelial cells (Ouslimani et al., 2007). Finally, metformin treatment of patient with T2D was associated with a decrease in the level of the soluble intercellular adhesion molecule-1 (ICAM-1) and the soluble vascular cell-adhesion molecule-1 (VCAM-1), both being directly correlated to increase in cardiovascular events in such population (De Jager et al., 2005). Interestingly, as for AGEs, this decrease in ICAM-1 and VCAM-1 level was independent of the antihyperglycemic action of the drug.

Metformin in Myocardial Injury

The protective action of metformin also occurs at the myocardium and cardiomyocyte levels. Several ex vivo experiments using perfused heart protocols revealed a protective action of metformin during an ischemic episode. In a working-heart perfusion model where metformin is administered before a mild ischemic episode, the antidiabetic drug improved rat cardiac functional postischemic recovery (Legtenberg et al., 2002). It has been also shown that metformin given at the time of reperfusion reduced myocardial infarct size in both nondiabetic and diabetic hearts (Bhamra et al., 2008; Paiva et al., 2009). In these two last studies, the protective action of metformin was associated with a PI3K-mediated inhibition of the mitochondrial permeability transition pore opening and with increased intracellular formation of adenosine. However, the same group also showed that chronic metformin treatment of diabetic rats augments their myocardial resistance to ischemia-reperfusion injury via a pathway involving AMPK activation and PGC-1α (Whittington et al., 2013). Similar results have been obtained using in vivo models of myocardial infarction. In mice subjected to permanent left coronary artery occlusion or to 1 hr left coronary artery occlusion followed by reperfusion, 4 weeks metformin treatment improved survival and preserved left ventricular dimensions and left ventricular ejection fraction (Gundewar et al., 2009). These effects were concomitant to AMPK and eNOS activation, to increase in PGC-1α expression and disappeared in mice lacking AMPK or eNOS. In a similar study, Yin and colleagues demonstrated that metformin treatment increases AMPK activity, improves cardiac function and reduces infarct size after a myocardial infarction in rats (Yin et al., 2011). This protective action of metformin was also established in a dog model of heart failure (Sasaki et al., 2009). The authors nicely showed that other AMPK activators, such as 5-aminoimidazole-4-carboxamide 1-β-D-ribofuranoside (AICAr), have the same effect than metformin, suggesting a role of this protein kinase. In cardiomyocytes, metformin reduced apoptosis by increasing antiapoptotic proteins and attenuating the production of pro-apoptotic proteins (Yeh et al., 2010). These effects correlate with AMPK activation and can be reproduced by AICAr (Yeh et al., 2010). Metformin and AMPK also influence autophagy, which is known to be dysregulated in diabetic cardiomyopathy and heart failure. Indeed, metformin is able to restore impaired autophagy via the dissociation of Bcl-2 from Beclin 1 in diabetic wild-type mice but not in cardiac-specific AMPK-dominant-negative transgenic diabetic mice (He et al., 2013; Xie et al., 2011).

Metformin in Cardiac Hypertrophy

Left ventricular hypertrophy is common among patients with T2D, who commonly have chronic hypertension. AMPK is a known inhibitor of cardiac hypertrophy via the negative regulation of protein synthesis (via mTOR inhibition) and of gene transcription including mitogen-activated protein kinase and calcineurin-nuclear factor of activated T cells pathways (Horman et al., 2012). In agreement with the antihypertrophic action of AMPK, metformin inhibits cardiac hypertrophy in a rat model of pressure overload (transverse aortic constriction) (Zhang et al., 2011). A very recent publication has reported that metformin protects against transverse aortic constriction-mediated cardiac hypertrophy independently of AMPK (Xu et al., 2014).

Metformin in Diabetic Cardiomyopathy

A healthy human heart produces around 5 to 6 kg of ATP each day to sustain its function, representing 20 times its own weight (Horman et al., 2012). This ATP is generated via the mitochondrial oxidation of fatty acids (for 70%) and glucose/pyruvate (for 30%). Fatty acids are preferred substrates because their oxidation inhibits glucose catabolism via the Randle cycle. However, in the postprandial state, insulin favors glucose utilization by promoting glucose uptake and glycolysis and inhibiting fatty acid oxidation. Currently, it is commonly accepted that this metabolic flexibility is essential for the maintenance of heart function. On the other hand, the metabolic inflexibility of diabetic hearts, which are insulin resistant and almost exclusively use fatty acids as source of energy, participates in the development of the diabetic cardiomyopathy (Bugger and Abel, 2014). Thus, therapies that promote glucose metabolism and normalize insulin sensitivity may reduce cardiac complications linked to diabetes. Metformin and AMPK are potential therapeutic candidates. Indeed, AMPK activation by metformin or other activators is able to stimulate cardiac glucose uptake and glycolysis independently of insulin, bypassing insulin resistance in insulin-resistant cardiomyocytes (Bertrand et al., 2006; Ginion et al., 2011). Even more interestingly, increase in AMPK activity by the same activators restored insulin sensitivity of insulin-resistant cardiomyocytes by a mechanism that still needs to be identified (Bertrand et al., 2006; Ginion et al., 2011). The concentration of metformin used in these acute (few hours) studies was, however, higher than those used in diabetic patients. We can postulate that lower concentrations of metformin could play similar role when utilized during longer time of exposure. In relation to the problematic of the effective dose of metformin in the heart, a new specific activator of AMPK (A-769662) has been recently shown to increase metformin sensitivity independently of the AMP/ATP ratio (Timmermans et al., 2014). Such compound might be useful clinically to increase metformin cardiac sensitivity.

On the other hand, similarly to the situation found in endothelial cells (Detaille et al., 2005; Ouslimani et al., 2005), hyperglycemia induced NADPH oxidase-mediated ROS production and cell death in cardiomyocytes (Balteau et al., 2011). It is tempting to

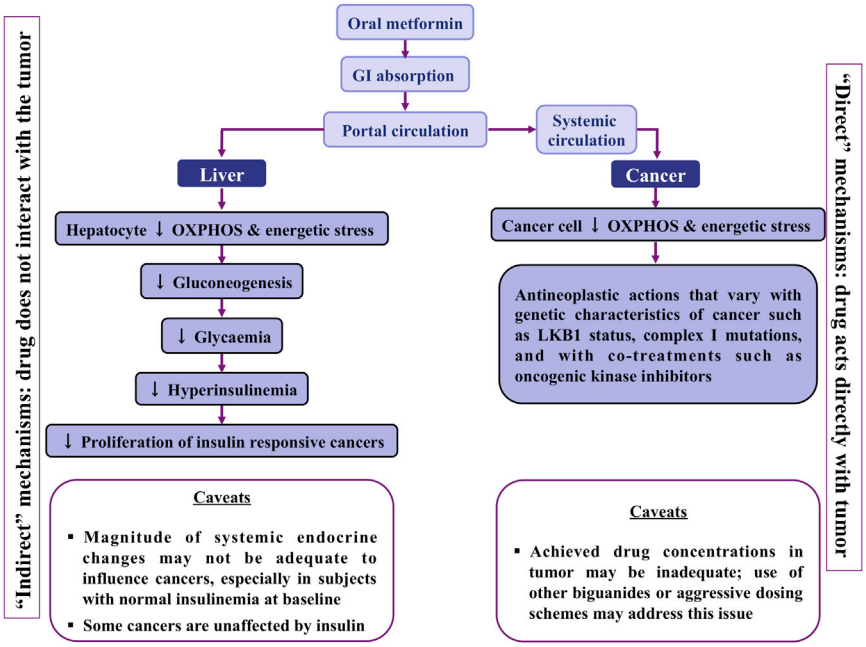
"Indirect" mechanisms: drug does not interact with the tumor
Oral metformin
GI absorption
Portal circulation
Systemic circulation
Liver
Cancer
Hepatocyte ↓ OXPHOS & energetic stress
↓ Gluconeogenesis
↓ Glycaemia
↓ Hyperinsulinemia
↓ Proliferation of insulin responsive cancers
Cancer cell ↓ OXPHOS & energetic stress
Antineoplastic actions that vary with genetic characteristics of cancer such as LKB1 status, complex I mutations, and with co-treatments such as oncogenic kinase inhibitors
"Direct" mechanisms: drug acts directly with tumor
Caveats
▪ Magnitude of systemic endocrine changes may not be adequate to influence cancers, especially in subjects with normal insulinemia at baseline
▪ Some cancers are unaffected by insulin
Caveats
▪ Achieved drug concentrations in tumor may be inadequate; use of other biguanides or aggressive dosing schemes may address this issue

Figure 3. Proposed Mechanisms by which Biguanides May Influence Cancer Biology

These mechanisms may be indirect, where the drug does not interact with the cancer cells but rather alters the endocrine-metabolic milieu of the host in a way that may influence cancers. Indirect effects include but are not confined to suppression of gluconeogenesis and decreased systemic glucose and insulin levels. The insulin-lowering effect of metformin is used as an example; other indirect mechanisms have been proposed. Direct interactions between biguanides and cancers are supported by recent experimental data and occur when drug exposure is adequate to induce energetic stress in cancer cells. There is uncertainty if conventional antidiabetic doses of metformin are sufficient to accomplish this. Genetic characteristics of a cancer likely influence not only the extent to which biguanides accumulate but also its ability to deal with energetic stress, and thus the degree of therapeutic benefit. Abbreviations: GI, gastrointestinal; LKB1, liver kinase B1; OXPHOS, oxidative phosphorylation.

speculate that AMPK activation by metformin would be able to reduce this ROS production and to increase cardiomyocyte survival under hyperglycemic condition as demonstrated in the endothelium (Batchuluun et al., 2014; Bhatt et al., 2013). In agreement with this hypothesis, it has been recently shown that AMPK activation by A-769662, the metformin analog phenformin, or the new antidiabetic drug GLP-1 limited glucotoxicity in adult cardiomyocytes (Balteau et al., 2014). The mechanism proposed in this study is the AMPK-dependent suppression of the hyperglycemia-mediated ROS production via the inhibition of the NADPH oxidase NOX2.

Metformin in Cardiac Fibrosis

Cardiac fibrosis is another element of diabetic cardiomyopathy and more generally heart failure. Interestingly, metformin is able to attenuate fibrosis in a canine model of heart failure, presumably via AMPK activation and its inhibitory action on transforming growth factor-β expression (Sasaki et al., 2009). Similar results were obtained in mice subjected to left ventricular pressure overload by transverse aortic constriction even if, in this case, AMPK did not seem to be involved (Xiao et al., 2010). In other studies, it has been established that metformin inhibits myofibroblast differentiation by suppressing ROS generation via the inhibition of the NADPH oxidase pathway (Bai et al., 2013), AMPK probably mediating this effect. Of note, a genetic link between AMPK and cardiac fibrosis has been recently demonstrated in a myocardial infarction mouse model (Noppe et al., 2014).

Antineoplastic Actions of Metformin

Pharmaco-Epidemiology

Pharmaco-epidemiologic evidence has played a major role in generating the hypothesis that metformin has utility in cancer prevention and/or treatment. A seminal report published in 2005 (Evans et al., 2005) presented evidence that diabetics treated with metformin had a substantially lower cancer burden than diabetics treated with other agents, and many other studies reached similar conclusions (Gandini et al., 2014). However, while results of certain studies are encouraging, there are contradictions in the available data, and the hypothesis remains controversial (Tsilidis et al., 2014). Obviously, the populations under study were type 2 diabetic patients, and the conclusions may not be applicable to nondiabetic subjects, even if the conclusions were validated for diabetics. More importantly, all these studies are based on retrospective reviews of medical records and are subject to a variety of potential biases (Suissa and Azoulay, 2014). On the other hand, some recent reports are consistent with the earlier pharmaco-epidemiologic data that provided evidence for reduced cancer burden among users of metformin. Examples include a study of multiple myeloma outcomes (Wu et al., 2014), one of prostate cancer risk based on the Danish cancer registry (Preston et al., 2014), and one regarding prostate cancer prognosis (Margel et al., 2013). Thus, pharmaco-epidemiologic data have played an important role in generating the hypothesis that metformin may be useful in cancer prevention or treatment but have not been consistent in supporting this concept.

Indirect Effects of Metformin on Cancer

The proposed mechanisms of action of metformin relevant to oncology can be divided into two broad, non-mutually exclusive categories: effects on the host that indirectly influence the cancer and direct effects on cancer cells (Pollak, 2012b) (Figure 3). As noted earlier in this review, direct actions of metformin on the liver inhibit hepatic glucose production, resulting in systemic metabolic and endocrine effects that may influence cancer biology. The most obvious candidate change of oncologic relevance is the reduction of hyperinsulinemia, given prior evidence that high insulin levels can stimulate proliferation of a subset of common cancers (Pollak, 2012a). Importantly, however, the magnitude of metformin-induced decline in insulin levels is greater in type 2 diabetics than in metabolically normal subjects, and it is not clear if metformin-induced changes in plasma insulin levels, particularly in nondiabetics, are sufficient to perturb tumor biology. Nevertheless, there is evidence from a murine model

that metformin administration is more effective with respect to tumor growth inhibition when diet-induced obesity and hyperinsulinemia are present, and it is associated with decreased activation of insulin receptors of cancer xenografts (Algire et al., 2011). Levels of various adipokines relevant to cancer biology are also influenced by metformin in models, but further clinical data in this area are required. Also, a few studies have suggested immunological or anti-inflammatory actions of metformin (Moiseeva et al., 2013; Pearce et al., 2009) relevant to oncology, but there are to date no clinical data to support or refute these observations. Thus, the role of "indirect" actions of metformin remains an active research topic.

Direct Effects of Metformin on Cancer

Dozens of in vivo and in vitro studies have shown direct antineoplastic activity of biguanides in model systems, but most of these have neither provided mechanistic details nor considered dose-response issues relevant to clinical applications (Pollak, 2012b; Viollet et al., 2012). One of these (Huang et al., 2008) provided evidence that AMPK activation is important in the action of biguanides by showing that the direct AMPK activator A-769662 (which does not inhibit OXPHOS) has in vivo antineoplastic activity. Other observations suggest the relevance of inhibition of respiratory-chain complex 1 within tumors underlies the therapeutic effect of metformin in vivo under conditions where dosing is adequate: a key finding was that tumor growth inhibition by metformin occurs under control conditions, but not when the tumor model is engineered to express the yeast metformin-resistant *Saccharomyces cerevisiae* NADH dehydrogenase NDI1 protein (Birsoy et al., 2014; Wheaton et al., 2014).

Alterations in cellular metabolism in a manner that is influenced by mutations in exposed cancer cells are important consequences of metformin-induced reduction of OXPHOS (Buzzai et al., 2007). This suggests the possibility of rational drug combinations (Ben Sahra et al., 2010). Particularly interesting work has provided a metabolic rationale for combining biguanides with small molecule drugs that inhibit oncogenic kinases that drive glycolysis (Pollak, 2013b). These studies support the possibility that cancer cells have a requirement to increase OXPHOS, at least transiently, to compensate for the decreased glycolysis that arises as a consequence of oncogenic kinase inhibition. In the presence of biguanides, this compensatory increase is attenuated, resulting in enhanced antineoplastic activity of the kinase inhibitor (Haq et al., 2013). Mutation of isocitrate dehydrogenase-1 (IDH-1) is an important topic in cancer metabolism, and there is preclinical evidence justifying study of the combination of a biguanide and inhibitors of mutated IDH-1 (Grassian et al., 2014). There is also evidence of a benefit of combining biguanides with conventional chemotherapy, radiotherapy, and hormonal therapies (Pollak, 2012b).

An early finding supporting a direct action of metformin on cancer cells was the observation that the drug was growth inhibitory in vitro in a manner that was associated with AMPK activation and mTOR inhibition as a consequence of metformin-induced energetic stress (Zakikhani et al., 2006). Further work in this direction showed that genetic ablation of the α1 catalytic subunit of AMPK accelerates Myc-induced lymphomagenesis, consistent with the observation that AMPK activation not only downregulates mTOR but also suppresses the excess aerobic glycolysis (Warburg effect) characteristic of most transformed cells (Faubert et al., 2013). This line of investigation suggests a tumor suppressor role for AMPK.

However, it is now recognized that AMPK activation under conditions of energetic stress can improve cell survival (Jeon et al., 2012) by tuning cellular energy metabolism to reduce energy consumption, in keeping with the evolutionary role of AMPK in adjusting metabolism to cope with low nutrient supply. Thus, it remains to be determined if the antiproliferative but prosurvival consequences of AMPK activation in cancer cells, either due to the inhibition of OXPHOS by metformin or the direct activation by specific pharmacological activators, will be of clinical benefit.

Consideration of the consequences of biguanide-induced energetic stress in cancer cells defective in AMPK activation is of particular interest. Xenograft (Algire et al., 2011) and transgenic (Shackelford et al., 2013) models have shown that cancers with loss of function of LKB1 are hypersensitive to biguanides. This observation is consistent with the notion that activation of AMPK, while associated with reduction in proliferation, actually mitigates energetic stress induced by metformin by reducing energy consumption, thereby favoring cell survival. Cancer cells functionally deficient in AMPK, in contrast, will be less likely to reduce energy consumption in face of biguanide-induced reduction in ATP generation and are therefore more likely to experience a lethal energetic crisis. This scenario is attractive because it implies a favorable therapeutic index (a greater effect on the cancer than the normal host tissues), as AMPK-defective cancers would be more sensitive to biguanides than normal tissues that retain a functional AMPK signaling system. This situation may be relevant to a sizable proportion of human cancers: for example, more than a third of non-small cell lung cancers are reported to be LKB1 defective. Cancers with mutations in genes encoding respiratory-chain complex 1 components have also been shown to be hypersensitive to biguanides (Birsoy et al., 2014).

Collectively, preclinical studies raise enthusiasm for clinical trials of metformin in a manner that is less controversial than the pharmaco-epidemiologic rationale. However, it is clear that precise mechanisms have not been defined, and it is important to point out that in order for metformin to have a direct antineoplastic effect, an adequate drug concentration must be achieved in neoplastic tissue. This concentration will be determined not only by the plasma level, which is related to the administered dose and to pharmacokinetic variables, but also to cellular uptake in cancer cells that may vary with respect to expression of OCT1 and other relevant transporters. The serum levels of metformin achieved in diabetic patients and in many in vivo models (as reviewed above) are in the micromolar range, while growth inhibition in vitro is usually observed at millimolar concentrations. Thus, an overarching research question is what metformin concentrations are achieved in neoplastic tissue of patients receiving conventional antidiabetic doses of metformin. Even before consideration of tumor biology, there may well be heterogeneity between cancer patients with respect to direct actions of metformin that arise as a consequence of whole-organism and cellular pharmacokinetic factors. Imprecision regarding achieved drug levels in target tissues in preclinical models that demonstrate antineoplastic

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activity makes extrapolation to the clinic hazardous: it remains uncertain if the laboratory data should be used to support clinical trials of conventional antidiabetic doses of metformin or clinical trials of more aggressive and novel methods of biguanide administration designed to maximize tumor drug concentration.

Clinical Advances and Outlook

While many clinical trials are ongoing, few actual clinical outcome results have been reported to date. However, there have been many publications of pilot studies that refer to surrogate endpoints such as changes in tumor proliferation rate between serial tumor biopsies obtained from patients prior to and during metformin exposure. Many of these have shown encouraging decreases in proliferation in breast, prostate, and endometrial cancers (Pollak, 2012b; Viollet et al., 2012), but overall results have been difficult to interpret because the magnitude of declines is in many cases smaller than those associated with currently approved treatments and because in some studies declines are confined to subsets of patients. It should be noted that the design of ongoing randomized clinical trials may not address some of the specific contexts suggested to be of high interest by preclinical work performed only after the trials were designed, such as strategic combinations of biguanides with tyrosine kinase inhibitors (Pollak, 2013b) or selective use of biguanides for tumors with respiratory-chain complex 1 mutations (Birsoy et al., 2014).

Anecdotal clinical evidence of stimulation of glucose uptake by intestinal mucosa by metformin has been presented in the nuclear medicine literature (Gontier et al., 2008). While from a radiologic perspective this was reported as a bothersome artifact, from a physiologic perspective the data may represent pharmacodynamic evidence of biguanide-induced inhibition of OXPHOS in intestinal mucosa, leading to energetic stress and a compensatory increase in glycolysis, resulting in the observed increase in glucose uptake. This may take place preferentially in bowel as compared to other tissues because of the high luminal concentration of metformin following oral administration, emphasizing the importance of pharmacokinetic factors in determining tissues where direct biguanide actions are likely to occur. These data are correlated with greater reductions of proliferation rate by metformin in colon (Pollak, 2013a) than in most other tissues examined, and reduced aberrant crypt foci in patients (Hosono et al., 2010), thus raising the possibility of specific applications for metformin in colorectal cancer prevention.

Ongoing research comprises two broad areas. One examines the hypothesis that metformin, when administered in the manner used for diabetes therapy, has utility for cancer prevention or treatment. There has been relatively little effort to address this possibility in a quantitative fashion in preclinical model systems because it is difficult to accurately simulate human pharmacokinetics following oral dosing in rodents, and because there has been enthusiasm among physicians to proceed directly to human studies (Pollak, 2014). Thus, there are more than 200 oncology clinical trials involving metformin in progress. Another research area, which has not yet led to clinical trials, involves optimization of biguanide pharmacokinetics to maximize exposure of neoplastic tissue. This work is based on one hand on the attractive laboratory data related to the "direct" mechanism of action and on the other hand on concern that conventional antidiabetic dosing may be inadequate for the desired effects. Relative to metformin, phenformin or novel biguanides (Narise et al., 2014) may have reduced requirements for cell surface transporters to enter cancer cells or other desirable pharmacokinetic properties. This has been clearly evidenced in rat hepatoma cells H4IIE, where quinidine, a competitive OCT1 inhibitor, completely blocked AMPK activation by metformin, while AMPK activation by phenformin and galegine was not affected (Hawley et al., 2010). Metformin may have very different effects if given parentally rather than orally. However, while such approaches may have advantages, they also may be associated with unacceptable toxicity, so that conventional preclinical work leading to phase I safety and dose-finding clinical trials would be required as a first step.

Conclusion and Therapeutic Perspectives

After more than 50 years of clinical experience, the utility of metformin in the control of hyperglycemia in T2D has been well established. The safety of metformin differentiated it from phenformin and buformin, which were withdrawn in most countries due to a higher risk incidence of lactic acidosis. The favorable risk/benefit profile of metformin has made it one of the most widely prescribed drugs in the world. Clinically, metformin exerts its antihyperglycemic effect mostly through the inhibition of hepatic gluconeogenesis. Its primary site of action is the mitochondria via its mild and specific inhibition of the respiratory-chain complex 1, thereby lowering energy charge and ultimately leading to a reduction in hepatic glucose output. Although the energy sensor AMPK is activated by a decrease in the cellular energy charge, it appears to be dispensable for the direct inhibitory effect of metformin on hepatic gluconeogenesis but still may indirectly inhibit by restoring hepatic insulin sensitivity. This is consistent with the role of AMPK in the lipid-lowering effects and improvements in insulin sensitivity by metformin in the liver. Recent advances revealed a new molecular target in the mitochondria with the direct inhibition of mGPD, resulting in altered cellular redox state and limiting lactate and glycerol contribution to hepatic gluconeogenesis.

The evidence presented in this review suggests that metformin may have clinical value in the treatment of cardiovascular complications associated with T2D by exerting a variety of cellular actions in different tissues and cell types. Chronic metformin treatment of patients leads to a basal state of cardioprotection, thereby potentially limiting the occurrence of myocardial infarction, heart failure, diabetic cardiomyopathy, and cardiac hypertrophy. Multiple molecular mechanisms were proposed, including reduction of ER stress, oxidative stress, apoptosis, and protein synthesis, as well as insulin resistance in endothelial cells, cardiomyocytes, and cardiac fibroblasts through AMPK-dependent and AMPK-independent pathways.

Some studies have raised the possibility that metformin may also be effective in providing protection against cancer. The concept of "repurposing" metformin for cancer prevention or treatment is appealing (Pollak, 2014), as the drug is inexpensive and well tolerated relative to commonly used antineoplastic agents. The original and most optimistic hypothesis, that this drug at conventional antidiabetic doses is useful for a wide variety of indications in oncology, was based in part on pharmacoepidemiologic data that are now considered controversial. As most experimental evidence for antineoplastic activity of

Table 1. Examples of Challenges in Biguanide Research

Elucidate the precise mechanism(s) by which metformin interacts with and inhibits the mitochondrial respiratory-chain complex 1
Clarify the effect of metformin on tissue-specific regulation of mitochondrial biogenesis and its subsequent impact on cellular energy homeostasis
Clarify if metformin actually acts as an ''insulin sensitizer'' in vivo or if the reduction of insulin level associated with use of the drug is completely accounted for by decrease in glucose secondary to decrease gluconeogenesis
Define mechanisms that limit duration of efficacy of metformin in treatment of T2D
Clarify why cardiac benefits of metformin are greater in diabetics than nondiabetics
Provide clinical evidence for or against clinical antineoplastic action of metformin in diabetic and nondiabetic cancer patients
Define optimum dosing of biguanides for cancer treatment in diabetic and nondiabetic patients, and establish if phenformin or novel biguanides offer advantages for cancer treatment
Delineate the role of AMPK in metformin action in different tissues and different disease states
Design and evaluate rational combinations of metformin with other pharmacological agents for various indications
Understand the genetic influences on efficacy of metformin for various indications and move toward personalized medicine to optimize therapies
Are recently described actions of metformin on the intestinal microbiome (Cabreiro et al., 2013) relevant to any of the clinical effects of the drug?

metformin involves drug exposure levels considerably higher than those in serum of metformin-treated diabetics, the use of novel metformin dosing strategies, including intravenous rather than oral administration, as well as the use of phenformin or novel biguanides designed to have pharmacokinetic characteristics optimized for oncologic indications may be worthwhile.

Furthermore, preclinical studies have identified not only rational drug combinations involving biguanides that deserve evaluation but also specific tumor characteristics, such as mutations of STK11/LKB1, that may be associated with biguanide sensitivity. Therefore, even if the first generation of metformin trials for cancer treatment is disappointing, there will be interesting questions to address in subsequent studies. On the other hand, demonstration of clinical benefit of any of the ongoing trials would represent an important example of productive ''repurposing'' research and lead to efforts to build on that success.

Among clinicians, metformin is regarded as a widely used and ''mature'' generic drug with a well-established and important role in treatment of T2D, for which there is a limited research agenda remaining. However, as reviewed here, from a mechanistic perspective, there are many important unanswered questions (Table 1) and a recognition that biguanides have unique therapeutic properties arising from their effects on cellular energy metabolism. It is possible that further understanding of the mechanistic aspects of biguanide pharmacology will result in advances in treatment not only of diabetes but also in cardiovascular and neoplastic diseases.

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